THE EFFECTS OF DIET ON THE INTESTINAL FLORA

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It has long been recognized that certain pathologic conditions in man are associated with an intestinal flora that is markedly putrefactive in type. Headaches, skin disorders, digestive disturbances, nonalcoholic cirrhosis, nervous abnormalities and cardio-vascular-renal disease have been attributed with more or less reason to the effects of excessive intestinal putrefaction. Metchnikoff [1] believed that premature senescence was the result of this bacterial activity, and the term "auto-intoxication" became almost a household word as the result of his theories. Many of Metchnikoff's assumptions were not founded on definitely established facts, and it has since been shown that in many of the cases of so-called "auto-intoxication" the abnormal symptoms were really the result of chronic infections in the digestive tract or elsewhere. When these foci of infection were removed the symptoms disappeared. In other cases the symptoms have been shown to be the result of nervous reflexes. After eliminating these cases, however, there are still many instances of acute and chronic conditions in man in which no focal infections have been found and in which there is definite evidence of an intoxication of intestinal origin.

It is generally assumed that these toxemias are due to the absorption of poisonous substances from the lumen of the intestinal tract and that these poisonous materials are formed as the result of the action of proteolytic bacteria on proteins or their split products. The presence of putrefactive products and of certain toxic amines in intestinal contents has long been known. Furthermore, it is well known that members of the colon group of bacteria have the ability in vitro of forming indol, skatol, phenol, hydrogen sulphide and certain toxic amines, such as histamine and tyramine. We know that some of these products are absorbed and excreted by the kidneys without any apparent ill effects. The evidence in regard to the absorption of toxic amines

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[1] The Prolongation of Life.

is not so clear, nor is it known to what extent the liver functions as a detoxicating organ when unusually large amounts of these substances are absorbed.

In certain acute conditions, such as intestinal obstruction, diarrheas and digestive disturbances in children, there is a considerable amount of evidence that these poisonous substances are absorbed to an unusual degree, and that the toxemia is the result of the increased absorption of these bacterial products. Dragstedt [2] and his coworkers have shown that in acute intestinal obstruction the presence of bacteria in the lumen of the intestine is necessary for the production of these toxic substances and that in their absence no poisonous materials are formed. In later work [3] it was shown that the toxemia incident to acute obstruction is uniformly associated with a proteolytic intestinal flora, irrespective of the nature of the flora before the obstruction was produced. In animals with an aciduric flora, experimental intestinal obstruction led to a very slowly developing toxemia as compared with animals in which a proteolytic flora was present at the time of operation. In diarrheal conditions it has been observed that the toxemia is profound when the diarrhea is of the putrefactive type, whereas in the fermentative type there may be little evidence of toxemia. Certain digestive disorders in children have been shown to be associated with a putrefactive intestinal flora. Morris, Porter and Meyer [4] have described such cases in which the flora was controlled by diet. These workers found that the return of these children to normal health was coincident with a regression of the intestinal flora toward predominantly fermentative types. The improvement in each case began at a time when it was possible to show that the intestinal flora had altered in type from putrefactive to fermentative.

Attempts to devise methods of preventing this putrefaction have been persistent and varied. Vaccines, intestinal antisepsis, removal of the colon, and implantation of antagonistic organisms have been tried with slight success. One popular procedure has been the use of lactic acid milks, particularly milk fermented by B. bulgaricus. The original belief was that this organism could be implanted in the intestinal tract and that it formed enough acid to inhibit the growth of the proteolytic types. As a whole, the use of this method has been disappointing and

[2] Jour. Exper. Med., 1917, 25, p. 421; 1919, 30, p. 109; Am. Jour. Physiol., 1918, 46, p. 366; Jour. Exper. Med. 1918, 27, p. 359.

[3] Cannon, P. R.; Dragstedt, L. R., and Dragstedt, C. A.: Jour. Infect. Dis., 1920, 27, p. 139.

[4] Jour. Infect. Dis., 1919, 25, p. 349.

within the last few years observations have shown that it is impossible to implant B. bulgaricus in the intestinal tract, since the organism is apparently not an inhabitant of the normal intestine and cannot adapt itself to its new environment.

The other method of lessening intestinal putrefaction is the substitution of carbohydrate food for protein, and within recent years many workers have clearly shown that under normal physiologic conditions the chemical character of the food ingested is the fundamental factor controlling the bacteria of the intestinal tract. This conception was advanced by Hirschler,[5] in 1886, when he found that in the case of dogs fed cane sugar, potatoes, glycerol and dextrin with meat, the feces contained indol and phenol in smaller amounts than in those fed meat alone. Winternitz,[6] a few years later, concluded that the feeding of milk tends to inhibit protein putrefaction and retards the formation of the protein split products. He attributed this influence to the lactose and believed that it was independent of the effect of the lactic acid. Through milk feeding, he claims, there is a decrease in the ethereal sulphates of the urine and a lessening of the last protein split products "worthless for the host and perhaps harmful."

Herter and Kendall[7] were the first (1908) to show clearly the effects of diet on the bacteria of the intestinal tract. They found that the intestinal flora of cats and monkeys was rapidly altered when a diet of meat and eggs was followed by one of milk and dextrose, there being a substitution of an aciduric nonproteolyzing type for one that was strongly proteolytic. In the same year Jungano[8] showed that the intestinal flora of white rats was changed when a meat die was fed, B coli then becoming the predominant organism although it had been absent or nearly so with the ordinary diet. De Gasperi[9] obtained about the same results as Jungano, finding that with an ordinary diet of wheat and bread the aciduric organisms were more prominent and with a meat diet the proteolytic bacteria appeared in great numbers.

Kendall has shown that in vitro proteolytic organisms are less active in the presence of carbohydrates, fermentation instead of putrefaction resulting. It is now a well-known physiologic fact that body cells and bacteria will obtain their carbon requirements from carbo-

[5] Ztschr. Physiol. Chem., 1886, 10, p. 306.
[6] Ztschr. f. Physiol. Chem., 1892, 16, p. 460.
[7] Jour. Biol. Chem., 1908, 5, p. 293.
[8] Compt. rend. Soc. de Biol., 1909, 66, pp. 112, 122.
[9] Centralbl. f. Bakteriol., I, O., 1911, 62, p. 519.

hydrates in preference to proteins and amino acids. It is only in the absence of carbohydrates that proteins are torn to pieces to supply energy. With this idea as a basis, several workers since 1908 have studied the effects of carbohydrate feeding on the intestinal flora. Sittler,[10] in that year, found that when lactose was fed to children on a cow's milk diet, B. bifidus became the predominant organism, and he concluded that the presence of B. bifidus in the intestines of breast-fed infants was due to the large amount of lactose in human milk. He showed that sucrose and levulose are unfavorable for the development of B. bifidus, while B. acidophilus becomes the predominating organism in infants fed malt soup.

Of the particular carbohydrates tested since that time, lactose and dextrin appear to be by far the most effective in transforming the intestinal flora. Distaso and Schiller [11] demonstrated with white rats that by feeding lactose or dextrin with bread and meat the intestinal flora could be changed into one consisting almost entirely of B. bifidus. They believed that these sugars arrived in the lower part of the intestines almost intact because of the lack of proper digestive ferments. When the sugars reached the region of bacterial activity the bacteria fermented them, and soon fermentative organisms controlled the field. Rettger and Horton,[12] in the same year, showed that when the diet of white rats was changed to one containing starch, lard, protein-free milk and a pure protein, the intestinal flora became much simpler and gram-positive organisms constituted from 85 to 100 % of the field as against 35 to 50 % with the ordinary mixed diet. Two organisms—B. acidophilus and B. bifidus—were frequently present to the exclusion of all other types. B. coli was also reduced in numbers in the special diet rats. Hull and Rettger [13] continued this work by studying the effects of various carbohydrates when fed to white rats with bread and vegetables. They used lactose, maltose, sucrose, dextrose, levulose, galactose, and dextrin. In the case of lactose B. bifidus soon became the predominating organism and often with few other bacteria accompanying it. The other carbohydrates gave negative results.

In a later publication [14] the same authors gave the conclusion of several years' study, namely, that a diet high in lactose was the most

[10] Ibid., I, O., 1908, 67, p. 14.
[11] Compt. rend. Soc. de Biol., 1914, 76, p. 243.
[12] Centralbl. f. Bakteriol., I, O., 1914, 73, p. 362.
[13] Ibid., 1914, 75, p. 219.
[14] Jour. Bacteriol., 1917, 2, p. 47.

effective in establishing an aciduric intestinal flora. Lactose in amounts of 1 to 2 gm. or more per day with a mixed diet led to a complete simplification of the flora in white rats. Milk and mixed grains tended to increase the number of aciduric bacteria, whereas meat increased the number of indol-producing bacteria. They also found that a high lactose diet in man led to the development of aciduric bacteria in large numbers. Torrey,[15] working with dogs, came to essentially the same conclusion as Rettger et al. Lactose and dextrin, when fed with a meat and rice diet, led to a marked replacement of proteolytic organisms by aciduric ones. Saccharose, maltose, and dextrose caused no pronounced change, although saccharose in large amounts caused a rise in the numbers of obligate fermentative bacteria. Starchy food led to a simplification of the intestinal flora and the development of B. acidophilus to a considerable extent. Vegetable proteins exerted a marked antiputrefactive tendency in certain cases. Mammalian tissues seemed to be the only ones which markedly encouraged the growth of putrefactive organisms in the intestines. Fish and casein did not encourage the development of putrefactive bacteria to the same extent as mammalian tissue. Fats exerted little effect on the flora.

The work here reported is based upon the idea that diet is the fundamental factor controlling the activities of the bacteria of the intestinal tract. The effort throughout the investigation has been to get a quantitative idea of the relative abundance of the different organisms, and with this end in view, particular attention has been paid to methods for the estimation of the numbers of bacteria of each type and the effects of diet on their presence.

METHODS

White rats have been used in this work because of the ease in handling, the small expense of feeding and because a large number of individuals can be used, thus ruling out a large part of the error due to individual idiosyncrasy. The feces have always been collected as far as possible in the manner described by Hull and Rettger. The rats were held by the tail and rubbed on the back above the base of the tail. In most cases the feces can be collected into tubes of sterile water or on clean papèr and are always fresh. These are then emulsified in 10 c c of sterile water and dilutions of 1:1000, 1:10,000 and 1:100,000 made. No effort has been made to weight out the feces as they vary so much in water content and residue in the various diets that only a fictitious accuracy would be attained. The effort throughout has been to determine the relative numbers of the different types rather than the numbers per unit weight of feces.

[15] Jour. Med. Research, 1919, 39, p. 15.

Torrey has shown that fecal bacteria in general may be conveniently grouped in accordance with their metabolic tendencies. According to this classification, there are two main groups composed of organisms which are predominantly fermentative or putrefactive in their tendencies. Intermediate between these are the members of the B. coli group which are either fermentative or putrefactive, depending on the nature of the food at their disposal. Members of this group are the predominant types of the aerobic intestinal bacteria in man and many of the lower animals, constituting about 60% of the viable bacteria of the feces, according to Kendall. In man at least their activities are usually more proteolytic than fermentative. B. acidophilus, on the other hand, is relatively abundant in children and is the predominant organism of the fermentative or aciduric group. Different foods cause a variation in the relative numbers of these two types, and this fact has been made the basis of the cultural work. By comparing the numbers of B. coli with those of B. acidophilus from the same fecal emulsion, a quantitative relationship can be shown. This relationship is expressed as the colon-acidophilus ratio or the C-A ratio. The following procedure has been followed in determining this ratio: The appropriate dilutions are made and carefully mixed and 1 c c portions added to sterile Petri dishes, using the same pipet in each case. The rule has been to make all platings in duplicate. To one set of plates the Ayers and Rupp [16] medium is added; to the other the beef liver glucose agar of Torrey.[17] The plates are incubated for 48 hours, and then the most favorable dilution for quantitative purposes is selected and the same dilution in the other medium is used with it. By using mediums adapted to these two types of organisms, other tvpes are ruled out to a certain extent, and a greater accuracy is thereby attained. All typical red colonies in the Ayers and Rupp medium are considered B. coli and all the small fluffy colonies resembling a fleck of cotton in the Torrey medium are considered B. acidophilus. These mediums are also prepared in small flasks and so the pouring of many plates is an easy matter.

The percentage of aerobes producing hydrogen sulphide in any fecal emulsion may be determined in a similar manner by using the acetate agar in plates. This medium gives a more accurate count than acetate broth and is as convenient to use. For finding the proportion of spore forming anaerobes, it is of great value. This is determined as follows: A tight plug of sterile absorbent cotton is pushed to the bottom of the tube of fecal emulsion, thus separating the solid particles from the bacteria. The supernatant fluid is divided into two portions, one of which is heated at 80 C. for 20 minutes. Dilutions of each portion are then made and plated in acetate agar, the unheated one aerobically and the heated portion by the Krumwiede-Pratt method. At the end of 36 hours both sets of plates are counted, and the proportion of spores to total viable aerobes in the same emulsion is found. B. welchii forms brown colonies in this medium and a good idea of the relative abundance of spores of this organism may be readily determined in this way.

STAINS OF THE FECAL EMULSIONS

Considerable information may be obtained from gram stains of the fecal emulsion. The study of smears in more than 50 instances in which the C-A ratio was 1-99 showed the uniform presence of from

[16] Jour. Bacteriol., 1918, 3, p. 433.
[17] Ibid., 1917, 2, p. 435.

75 % to 99 % of slender gram-positive bacilli. In a great many cases in which the animals were on a diet of bread, milk and lactose, the fecal smears showed practically 100 % of slender gram-positive bacilli in bunches and palisade arrangement. On the other hand, if the flora is proteolytic in type, there is a great preponderance of gram-negative bacilli, spirilla and large gram-positive spore-forming bacilli.

MEDIUMS

The following mediums have been used and incubation at 37 C. has been the rule in all cases.

1. *Ayers and Rupp Agar.*—This medium is of distinct value in determining the numbers of B. coli and in that way ascertaining the effects of the diet on their relative abundance. The fact that the readings are made at the end of 48 hours makes this medium valuable in conjunction with Torrey's beef-liver agar. The reliability of this medium in determining the presence of B. coli is shown by the fact that of 152 colonies transferred to peptone broth, 149 formed indol, thus showing their proteolytic powers.

2. *Beef Liver Glucose Agar.*—I have found this solid plate method well adapted for the quantitative estimation of B. acidophilus. With an initial reaction of P_H 5.7 to 6.0 the characteristic fluffy colonies are well developed at the end of 48 hours and are easily counted.

3. *Lead Acetate Agar.*—This medium has been useful in getting a quantitative idea of the presence of hydrogen-sulphide formers, both aerobic and anaerobic. This is a 2% agar prepared from beef infusion, containing 3% of peptone and 0.5% of NaCl. The reaction was P_H 7.4 to 7.8. Armour's peptone has been the best of the peptones tried and has been used throughout the experiments. Just before the plates are poured, 2% of sterile 10% lead acetate in distilled water is added to the medium, which has been cooled to about 50 C. and thoroughly mixed. This medium is prepared in small flasks (250 c c), which facilitates the pouring of a large number of plates at one time. After the medium in the plates is hardened, about 15 c c of sterile 3% agar in water is poured over the surface. This prevents the growth of surface spreaders and also leads to a slight reduction in oxygen tension which apparently favors the production of hydrogen sulphide. In the plates the hydrogen sulphide producing organisms appear as dark brown colonies, in some instances with a brown zone surrounding them. By comparing the number of these colonies with the total number of colonies on the plate, the relative abundance of the aerobic hydrogen-sulphide-producing organisms may be ascertained. A great variety of organisms develop in this medium, including B. acidophilus, and apparently the total number of viable aerobic bacteria in any particular fecal emulsion may be quite accurately determined. In the same manner the relative proportion of spore-forming anaerobes may be found, using the Krumwiede and Pratt method of pouring the medium into the cover of the Petri dish, placing the bottom on it and sealing the edge with sterile paraffin.

4. *Sheep Brain Medium.*—This medium is prepared according to the method of Hall[18] and is useful in determining the presence of putrefactive spore-forming anaerobes. These forms cause a distinct blackening of the medium,

[18] Jour. Infect. Dis., 1920, 27, p. 579.

as B. sporogenes for example, within 24 hours. By heating any particular fecal emulsion at 80 C. for 20 minutes, making dilutions in sterile water and inoculating 1 c c portions into tubes of the sheep brain, the presence and relative abundance of putrefactive types may be ascertained.

COLON-ACIDOPHILUS RATIO

The object of this work was to test out the methods described to determine the quantitative relationships of the two groups under the influence of certain diets. Rats were fed a stock diet of oats and carrots, ground beef, American cheese, meat and dextrin, and bread, milk and lactose. In addition, the effects of a diet high in lactose, dextrin, and vegetable protein were tested on three adults.

Oats and Carrots.—Rats on this stock diet for 2 weeks showed a large proportion of slender gram-positive bacilli in stains from the fecal emulsions. The colon-acidophilus ratio, the average of 1 plating from 4 rats, was 1:99.

Ground Beef.—The C-A ratio of rats on a high animal protein diet consisting almost exclusively of ground beef was 80:20. This figure is the average of 35 platings from 23 different animals which had been on the diet from 5 to 30 days. In older animals and in those that have been on the meat diet longer the C-A ratio rises, in many cases being 99:1. The stains from the fecal emulsions show a great preponderance of gram-negative organisms, the emulsions as a rule are foul smelling and give a much stronger indol reaction than do those of rats on either the stock diet of oats and carrots or bread, milk and lactose.

American Cheese.—Four rats were put on a bread, milk and lactose diet for 2 weeks. White bread was soaked in whole milk and mixed with lactose in the proportion of approximately 2 parts of bread to 1 of lactose. Their average C-A ratio at the end of this time, the result of 2 sets of platings, was 15:85. The rats were then fed only American cheese, with a small amount of carrot and cabbage mixture. At the end of 4 days the C-A ratio was 65:35, after 2 weeks 85:15, and at the end of 1 month 90:10. The diet was then changed, white bread and milk being substituted for the cheese. At the end of 1 week the C-A ratio was 6:94. These figures indicate that the relative abundance of proteolytic and aciduric organisms can be altered markedly in proportion to the presence of an abundant protein or carbohydrate diet.

Dextrin.—Torrey found dextrin even more effective than lactose in bringing about the establishment of an aciduric flora in dogs, the fecal flora being dominated by B. acidophilus. Hull and Rettger with white rats found dextrin effective in only a few instances, and in these the flora was not markedly simplified nor did B. acidophilus appear in great prominence. In later studies, however, Cheplin and Rettger [19] found dextrin as effective as lactose in stimulating the proliferation of B. acidophilus in white rats. My experience has been the same, dextrin leading to a marked simplification of the intestinal flora Four rats were fed only ground beef, carrots and cabbage leaves for 3 weeks Their average C-A ratio at the end of this time was 91:9. Dextrin and ground beef, 3 parts of dextrin to 7 parts of meat, were then fed. Four days later the average C-A ratio was 2:98, B. acidophilus having completely gained the ascendency. The feces were light yellow in color with the dextrin diet,

[19] Abstr. of Bacteriol., 1920, 1, p. 8.

whereas they were black with the full meat diet, and the indol reaction in the former case was faint or absent. Streptococci were also increased in numbers in this diet, and the hydrogen-sulphide producing bacteria were practically eliminated.

Lactose.—This sugar, when added to a diet of bread and milk in the general proportions given, caused a striking change in the character and flora of the feces. Here, too, the feces were softer and a lighter yellow than those of the meat eaters; there was less odor, and the indol reaction with the fecal emulsion was slight and in many instances negative. Culturally the C-A ratio the average of 29 platings from 18 different rats that had been on this diet from 5 to 30 days was 1:99. The stains from the fecal emulsions showed a great preponderance of slender gram-positive bacilli.

EFFECT OF LACTOSE ON MAN

The effect of a diet of milk, toast and lactose was determined in the case of 2 adults. For a period of 10 days each person consumed one half pound of lactose daily in addition to the regular diet of milk and toast. On an average 800 gm. of bread (toasted) and 1 liter of whole milk was consumed daily by each. Little butter was used, not more than 10 gm. per day. H-2 also consumed 2 liters of sour milk daily during the 3d, 4th and 5th days of the experiment, and then this was discontinued. The lactose was suspended in milk or water and a little vanilla added to make it more palatable. The fecal specimens were obtained after epsom salts had been given, and presumably represented a somewhat higher level of the intestinal tract than if this had not been done. Dilutions were immediately made and plated in plain infusion agar, beef-liver agar, and Ayers and Rupp agar. The large amounts of lactose caused considerable abdominal distention at first, but during the last 3 days of the experiment this was not so noticeable.

H-1: At the beginning of the experiment the flora of this person, as shown by cultural tests, was typical of the average adult on a mixed diet. B. coli was the predominant organism, and stains showed a field which was composed predominantly of gram-negative bacilli with a few plump gram-positive bacilli and diplococci. At the end of 3 days there was a slight increase in the number of gram-positive bacilli and diplococci. At the end of 6 days, B. coli was still the predominant organism culturally but the slides showed many slender gram-positive beaded bacilli, singly and in filaments. At the end of 10 days about 50% of the organisms in the smears were these slender gram-positive beaded bacilli. The cultural tests were unsuccessful, so it is uncertain whether these were B. acidophilus or B. bifidus.

H-2: The flora of this person at the beginning of the experiment was similar to that of H-1, gram-negative bacilli being in predominance both culturally and in the stained smears. At the end of 3 days there was a slight increase in the number of slender gram-positive bacilli and diplococci. Two liters of sour milk were consumed daily during the next 3 days, and at the

end of this period about 59% of the organisms in the stains were gram-positive diplococci. Culturally about 95% were streptococci, presumably Streptococcus lacticus. The sour milk feeding was then discontinued, and at the end of the 10th day of the experiment the streptococci were practically gone. At this time about 50% of the organisms in the stained smears were slender gram-positive beaded bacilli. B. acidophilus appeared on the beef-liver plates, although here B. coli was in predominance. The disappearance of the streptococci after discontinuing the ingestion of the sour milk illustrates the difficulty of implanting foreign organisms in the intestinal tract.

H-3: The effects of a diet relatively high in vegetable protein were determined in an adult. This man consumed during a period of 10 days 29 meals, each consisting of 100 gm. of "black-eyed peas," 400 gm. of white bread (toasted), 10 gm. of butter and a small amount of lettuce. This furnished approximately 2,700 calories daily and was considered adequate for a student leading a sedentary life. Before the diet was begun the normal flora was determined by plating in acetate agar aerobically and anaerobically, beef-liver agar and Ayers and Rupp medium. In each case the feces were collected after the administration of epsom salts.

At the beginning of the experiment the fecal emulsions were typical of an adult on a fairly high animal protein diet. The stains showed a great predominance of gram-negative bacilli and many large plump gram-positive bacilli. Aerobic hydrogen-sulphide-producing organisms were not prominent, but anaerobic spore-forming organisms were in great abundance and consisted almost exclusively of B. welchii. (Thirty-seven separate brown colonies from the anaerobic plates were picked to litmus milk and incubated anaerobically. All gave a typical "stormy fermentation" with butyric acid odor. These were transferred to dextrose broth and dextrose agar and found to be anaerobic nonmotile bacilli. Furthermore, the 3-day milk cultures were heated at 80 C. for 20 minutes and then 1 c c portions were transferred to fresh litmus milk and incubated anaerobically with no growth apparent at the end of 10 days' incubation.) The ratio of viable aerobes to anaerobic spores in the same fecal emulsion before the diet was begun was approximately 5:1. The predominant organism in both the acetate agar and beef-liver agar was B. coli.

At the end of 4 days of the diet there was a pronounced change in the bacterial content of the fecal emulsions. At this time a streptococcus began to appear prominently in both the acetate agar and beef-liver agar plates, apparently replacing B. coli. Also, there was an enormous decrease in the relative numbers of anaerobic spores so that the ratio of viable aerobes to spores was 20,000:1. The smears consisted predominantly of gram-negative bacilli, although there was an increased proportion of slender gram-positive bacilli and diplococci.

This streptococcus was the predominant organism of the aerobes at the end of 7 days, and was found to ferment dextrose, lactose, maltose and mannite. Presumably it belonged in the streptococcus fecalis group as described by Oppenheim.[20] B. welchii was practically suppressed and remained so for the remainder of the experiment.

At the end of 10 days the flora was predominantly fermentative with the streptococcus dominating the field and the anaerobic spores eliminated, as shown by the fact that in a fecal emulsion containing 25 000,000 viable aerobes per c c there was less than 100 per c c of anaerobic spores. The stains showed gram-positive streptococci and slender bacilli occupying about 50% of the field, with only an occasional plump gram-positive bacillus present.

[20] Jour. Infect. Dis., 1920, 26, p. 117.

The diet was discontinued at this point, and a diet high in animal protein consumed. At the end of 3 days a fecal specimen collected as before was examined. B. coli was once more in predominance, and B. welchii was again present in large numbers in the anaerobic plates. The stains showed many plump gram-positive bacilli in a field which was composed predominantly of gram-negative bacilli.

HYDROGEN-SULPHIDE PRODUCTION

Hydrogen sulphide is formed in the intestines by the decomposition of cysteine and cystine. It is claimed by some (Mathews) [21] that this is readily reabsorbed and produces headaches and depression even when absorbed in small quantities. This author also suggests that it may be one of the factors in hemolyzing erythrocytes, thus causing anemia in those suffering from constipation. Mercaptans, such as ethyl and methyl mercaptan, are very ill smelling compounds that are also formed by the action of certain intestinal bacteria. Acute pathologic conditions due to the action of these substances appear to be quite rare, and with the exception of a case of intoxication with hydrogen sulphide of intestinal origin described by Senator,[22] no definite cases have been reported. Van der Bergh, however, has shown the presence of sulphemoglobin in the blood of persons with intestinal obstruction.

Torrey in his investigations found that members of the proteus group are very active producers of hydrogen sulphide, and that they were more abundant in the ileum than in any other part of the intestinal tract in the case of a dog on a lactose-meat-rice diet. He also found that saccharose, maltose and dextrose exercised some anti-putrefactive action, as evidenced by the decrease in the number of hydrogen-sulphide-producing organisms and B. welchii. These sugars were not as effective as lactose and dextrin, however. With a high animal protein diet (beef heart) the hydrogen sulphide producers, especially B. proteus, became numerous, and with a fish diet they seemed to be brought to development to a greater degree than with any other food element.

By the use of the lead acetate agar described, a marked difference in the relative abundance of these hydrogen-sulphide formers with different types of diet may be shown. Table 1 illustrates the difference in numbers with two diets, expressed in percentage of hydrogen-sulphide producing-organisms to total viable aerobes in the same fecal emulsion. One lot of 5 rats was fed a meat and carrot diet, while the

[21] Physiological Chemistry, Text-Book, 1915, p. 443.
[22] Wells, Chemical Pathology, 1918, p. 581.

other lot received the same diet with the addition of lactose in the proportion of meat 7 parts to lactose 3 parts. The platings were made after the animals had been on the diets for at least 2 weeks.

It is evident from table 1 that there is a material decrease in the number of hydrogen-sulphide producing organisms in rats on a meat and lactose diet.

The most typical brown colonies developing on the acetate-agar plates are members of the proteus group. Certain strains of B. coli also form distinct brown colonies at the end of 36 hours. Mr. R. W. Cooper and Miss Mirium Jackson made a somewhat detailed study of this point with the following results: Forty-four strains picked from

TABLE 1

NUMBER OF HYDROGEN-SULPHIDE FORMERS PRODUCED WITH TWO DIETS

Meat Diet			Meat and Lactose Diet		
Rat	Total Bacteria per C c	H_2S Formers per C c	Rat	Total Bacteria per C c	H_2S Formers per C c
49	5,240 000	80,000	34	6,400,000	10,000
1.	360 000	30.000	6.	890,000	10,000
2.	80 000	20,000	7.	800,000	1,000
3.	310 000	10 000	8.	11,600	100
4.	3,700,000	70,000	9.	360 000	20,000
5.	610,000	140,000	10.	1,000 000	100
			6.	50,000	8,000
			7.	25,000	1,200
			8.	35 000	100
			9.	25,000	100
			10.	32,000	200
Total	10,300,000	350,000	....	9,628,600	50,800
Percentage	3.4		....	0.5	

acetate-agar plates from different rats on various diets were B. proteus vulgaris, giving the typical spreading growth on agar slants, liquifying gelatin, forming indol, and fermenting maltose, saccharose and galactose, with gas, but negative to lactose, mannite and inulin. One hundred brown colonies in plates from rats fed meat or salmon were picked and 50 were identified as B. proteus. On the other hand, the predominant brown colonies in plates from rats fed lactose and bread or egg yolk were members of the colon group. Ninety-four strains of the latter group were studied and found to be about evenly divided between saccharose-fermenting and saccharose nonfermenting types. In no case, however, was the formation of hydrogen sulphide as active as with the members of the proteus group.

Anaerobes.—No extensive analysis of the spore-forming anaerobes found in the intestinal tract of the white rat or man has been made in

the present study, but certain suggestive facts have been determined. For example, hydrogen-sulphide-producing anaerobes are found in large numbers in the feces of white rats when on a high animal protein diet and are greatly reduced in numbers or practically completely eliminated when lactose, certain vegetable proteins or starches are fed. These bacteria appear to be B. welchii in the majority of cases, as evidenced by the following experiments: Well separated brown colonies from the anaerobic plates have been transferred to litmus milk and incubated anaerobically. Eighty-nine such colonies from rats fed either meat, beans, egg yolk, milk or bread and lactose when picked to litmus milk gave typical stormy fermentation with butyric acid odor in 24 hours. About 75 of these were transferred to dextrose agar and shown to be anaerobes. Forty of those in dextrose broth were nonmotile by the usual motility test, of 42 tested. Twelve dextrose broth cultures (48 hours) were heated to 80 C. for 20 minutes and 1 c c portions added to litmus milk. Ten gave no growth in 10 days' incubation, while the cultures which showed motile bacilli gave a soft coagulum with a clear whey and no gas with a gradual peptonization of the curd. Nineteen milk cultures showing stormy fermentation (3 days' old) were heated in the same manner and 1 c c portions transferred to litmus milk. Seventeen gave no growth at the end of 10 days, while 2 coagulated the milk with a soft curd and peptonization.

From these tests it was concluded that in the white rats tested B. welchii was the most prominent of the spore-forming anaerobes, accompanied in smaller numbers by B. sporogenes.

The effects of the following food stuffs when fed alone for several days to white rats have been determined with reference to the development of hydrogen-sulphide producing organisms; ground beef, whole eggs, egg white, egg yolk, cheese, potato, lima beans, cow peas, English peas, salmon, bread, milk and lactose, and adiabetic flour (Hepco). These foods were fed in rotation to a series of rats, and the relative numbers of hydrogen-sulphide-producing organisms determined for each diet. The averaged results are:

Salmon: Four rats were fed salmon for from 1 to 4 weeks. The average of 12 platings during this period showed that 7.3% of the organisms growing on the acetate agar aerobically were producers of hydrogen sulphide. Approximately 200,000,000 colonies were counted in getting this percentage. These results agree with those of Torrey in that these hydrogen-sulphide-producing organisms were almost entirely B. proteus vulgaris. This fish diet also led to an enormous increase in the numbers of spore-forming anaerobes, especially B. welchii.

Ground Beef: Seven rats were fed ground beef for from 2 to 19 days. The average of 16 platings during this period gave a percentage of 7.5 for hydrogen-sulphide-producing organisms out of about 50,000,000 colonies counted. Hydrogen-sulphide-producing anaerobes were abundant in this diet, and of the spore formers B. welchii was predominant, although no more so than in the case of the fish diet.

Diabetic Flour (Hepco): This flour, according to the manufacturer, has as its base the Soya bean and contains approximately 43% protein, less than 23% carbohydrate with only a trace of starch, 21% fat and about 4.5% each of water, ash and fiber. This flour was fed uncooked to 7 rats for a period of from 2 to 7 days. The average of 8 platings from these animals showed that 0.05% of 50,000,000 colonies were hydrogen-sulphide producers. B. acidophilus was in predominance in all the plates and spore-forming anaerobes were almost completely suppressed. Even when this flour followed a diet that had led to a greatly increased percentage of B. welchii, the Hepco flour led to the elimination of B. welchii spores or reduced their numbers to a negligible quantity.

Cow Peas: The foregoing results with a diet high in vegetable protein suggested that perhaps this was a property of vegetable proteins in general, and although the legumes have almost 3 times as much carbohydrate as protein, nevertheless is was considered advisable to test out their effects as units on the intestinal flora. Cow peas which had been autoclaved at 15 pounds pressure for 20 minutes were fed to 5 rats for from 2 to 8 days. The platings during this period gave a percentage of 0.03 of hydrogen-sulphide-producing organisms out of a total of more than a billion colonies considered. This diet brought B. acidophilus in marked predominance to as great a degree as did the diet of meat and lactose. The spore-forming anaerobes were also completely suppressed by this diet, as for example in certain fecal emulsions in which there were 150,000,000 viable aerobes per c c with less than 100 spores of B. welchii per c c.

Lima Beans: These were also autoclaved in an equal volume of water at 15 pounds pressure for 20 minutes and fed to 3 rats for from 6 to 8 days. The average of 6 platings during this period showed that 0.03% of the colonies developing were producers of hydrogen sulphide. Approximately 350,000,000 colonies were considered in getting this percentage. Here too, B. welchii and other spore-forming-anaerobes were practically eliminated.

English Peas (Canned): These were fed to 4 rats for 6 days and platings made at the end of that period. The average of this series gave a percentage of 0.2 of hydrogen-sulphide producers. This percentage was somewhat higher than with the other legumes largely due to the fact that rat 18 had a flora containing an unusually high proportion of B. proteus. Even in this rat, however, B. welchii was completely suppressed as it was in the case of the other 3 animals on this diet.

Potatoes (White): These were autoclaved at 15 pounds pressure for 20 minutes and fed to 5 rats for from 2 to 4 days. The average of 8 platings gave a percentage of 0.4 of hydrogen-sulphide producing organisms. The percentage at the end of 4 days of the diet was less than this, indicating that the high starch diet tended to eliminate hydrogen-sulphide producers. B. welchii and other spore-forming anaerobes were almost completely suppressed at the end of 4 days.

Egg Albumin: Six rats were fed coagulated egg white exclusively for from 4 to 10 days and gave an average of 3% of hydrogen-sulphide producers out of 200,000,000 colonies considered Anaerobic spores were greatly increased

in numbers. Three of the rats on this diet died within 5 days, one in 6 days and another in 10 days. The rats were in separate cages, and as no deaths occurred with the other diets during a period of 2 months, it appears probable that there must have been some toxic effect from the unbalanced diet of egg white.

Egg Yolk: One rat only was tested. This animal was fed boiled egg yolk for one month. During this period 5 platings gave a percentage of 3.2 of hydrogen sulphide producers in 30,000,000 colonies examined. Anaerobic spores were increased in numbers also.

Whole Eggs: Six rats were fed scrambled eggs for from 4 to 12 days. Seven platings during this period gave 2% of hydrogen-sulphide formers. Anaerobic spores were also present in enormous numbers with this diet.

American Cheese: Six rats were fed cheese for from 2 to 3 days. Anaerobic spores were practically eliminated with this diet. The aerobic producers of hydrogen sulphide were present in 0.9% of the colonies counted. This is somewhat similar to the findings of Torrey that both casein and butter fat had little tendency to encourage the growth of putrefactive types.

TABLE 2

C-A RATIO OF VARIOUS REGIONS

Rat	Diet	Stomach C-A Ratio	Duodenum C-A Ratio	Jejunum C-A Ratio	Ileum C-A Ratio	Cecum C-A Ratio	Colon C-A Ratio
A	Lactose, bread and milk	0*	1-99	1-99	1-99	1-99	15-85
B	Meat	0*	0*	0*	99-1	99-1	99-1

* Less than 100 organisms per c c of emulsion.

Bread, Milk and Lactose: Seven rats were fed this diet for from 2 to 10 days. Equal parts of dried bread and lactose were soaked in milk and fed. The platings during this period gave a percentage of hydrogen sulphide producers of 0.06 out of 500,000,000 colonies considered. Anaerobic spores were almost completely eliminated by this diet.

INTESTINAL SURVEYS

Two rats, one on a diet of ground beef for 2 months and the other on a bread, milk and lactose diet for 2 weeks, were killed and under aseptic conditions dilutions of the contents of the stomach, duodenum, jejunum, ileum, cecum and colon plated to get an idea as to the relative distribution of B. coli and B. acidophilus. The emulsions from the different levels were adjusted as nearly as possible to the same turbidity to make the results somewhat comparable. The C-A ratio of the various regions is shown in table 2.

In the case of the animal on the high animal protein diet, we find both B. coli and B. acidophilus present in extremely small numbers in the stomach, duodenum and jejunum. B. coli appeared in large numbers in the ileum and was the predominant organism from there on. Torrey has noted that putrefaction, as judged by types and biologic activities of the bacteria, was more marked in the ileum than in the large intestine, and he suggested that this might be the case in persons suffering from toxemias of intestinal origin, to whom colonic irrigations afforded no relief.

The findings were reversed with the rat on a high lactose diet. Here we find large numbers of B. acidophilus appearing in the duodenum (80,000 per c c

as against less than 100 of B. coli from the same emulsion) and being in enormous predominance throughout the remainder of the intestinal tract. The emulsions from this animal were also plated into acetate agar. In the case of 1 c c amounts of the original emulsions from the stomach, duodenum and jejunum, not a brown colony appeared on the plates. A few colonies were present in the ileum, cecum and colon, but their number was insignificant when compared with the total number of B. acidophilus per c c in the same emulsions. In both cases, however, the first evidences of activity of proteolytic bacteria were found in the ileum.

Rat C. was fed lactose, bread and milk for 8 days. At the end of 5 days the feces were soft, a light yellow, practically odorless and showed no trace of indol. Stains showed a field composed almost entirely of slender gram-positive bacilli and a few short chains of streptococci. On the eighth day the animal was killed, and the various regions plated to determine the distribution of hydrogen sulphide producing organisms. Here B. acidophilus was found in large numbers from the duodenum on, while B. coli was practically absent. Hydrogen-sulphide formers were also in insignificant numbers throughout the intestinal tract.

DISCUSSION

A contrast in the tendency of animal and vegetable proteins to encourage putrefaction in the intestinal tract is clearly shown in these experiments. Torrey pointed out this peculiarity when he found that "vegetable proteins do not offer the slightest encouragement to the growth of the intestinal putrefactive types of bacteria." My experiments agree with those of Torrey in that vegetable proteins not only reduced the relative proportion of proteolytic types both aerobic and anaerobic, but also encouraged the overgrowth of a nongas-producing aciduric flora. Animal proteins, on the other hand, such as meat, fish and eggs, led to an enormous overgrowth of gas-forming proteolytic types.

The question of the absorption and excretion of materials from the intestinal tract has been recently studied by Underhill and Simpson.[23] These workers have found that the diets which give rise to the excretion of phenol and indican in large amounts are the ones that lead to the overgrowth of putrefactive bacteria in the intestinal tract. Meat led to a marked increase in the excretion of phenols and indican, whereas casein caused much less phenol and indican to appear in the urine. Lactose in the diet caused the excretion of phenol and indican to be lower than when large amounts of protein were fed. They found vegetable proteins to be on the same level as casein in regard to the excretion of phenol and indican. This work is a further indication that there is a definite correlation between the formation and the absorption of the by-products of bacterial activity in the intestinal

[23] Jour. Biol. Chem., 1920, 44, p. 69.

tract. It may be more than a coincidence that this increased absorption accompanies a gas-producing type of flora. It is a well-known fact that absorption in the intestine is increased by raising the intra-intestinal pressure, independently of the increase in mucosa surface. In an intestinal tract distended with gas both factors may be concerned and lead to an increased absorption that may be concerned in the production of mild or acute grades of toxemia. As pointed out, the detoxicating capacity of the liver is limited in acute intestinal obstruction, and there is evidence that it may be limited in some of the subacute and chronic toxemias that are apparently of intestinal origin.

SUMMARY

In this paper certain methods for ascertaining the relative proportions of groups of bacteria of the intestinal tract are described, particularly in studying the hydrogen-sulphide-producing organisms and the spore-producing anaerobes. By the use of these methods essentially the same results as those of Kendall, Rettger et al and Torrey have been obtained, namely:

Grain foods, lactose and dextrin when fed to albino rats in proper proportions lead to a marked predominance of aciduric bacteria in the intestinal tract, whereas animal proteins encourage the gas-producing proteolytic types, both aerobic and anaerobic.

Vegetable proteins and certain starchy foods do not encourage the development of proteolytic types to the same extent as animal proteins, and, in fact, in many cases exert a distinct antiputrefactive effect, favoring the development of B. acidophilus and suppressing the development of hydrogen-sulphide-producing organisms and spore-forming anaerobes.

In 2 experiments with human adults extending over a period of 10 days a diet composed of bread, milk and lactose markedly encouraged the development of the aciduric organisms, and in one experiment of the same time period a diet high in vegetable protein led to a predominant aciduric flora with the elimination of anaerobic spores.